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14. ABSTRACT

The deregulation of cell cycle checkpoints, with loss of regulation at the G1/S transition, has been shown to play an important role in the transformation to a malignant phenotype. Our studies have focused on cyclin E, which appears in late G1 and flanks the restriction point .We hypothesize that alterations of cyclin E in ovarian cancer cells contributes to the oncogenesis of ovarian tumors and negatively impacts outcome in patients with Stage I-III cancer. In this proposal we will i) develop a comprehensive ovarian cell line model for characterization of the role of cyclin E in ovarian cancer, ii) delineate the role of cyclin E and its tumor specific LMW forms in the development of malignant phenotype in vitro and in nude mice. iii) establish the prognostic value of the hyperactive forms of cyclin E in patients with Stage I-III ovarian cancer and iv) examine the biochemical significance of the LMW forms of cyclin E in tumor specimens. The results from our studies will provide much needed information about the molecular biology of ovarian carcinoma and may open new avenues for the development of targeted therapies.

15. SUBJECT TERMS
Ovarian cancer, cyclin E, LMW forms, predictive marker

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Introduction:

The overall purpose of this 3 year study is to test the hypothesis that alterations of cyclin E in ovarian cancer cells contributes to the oncogenesis of ovarian tumors and negatively impacts outcome in patients with Stage I-III ovarian cancer.

Over expression of the cyclin E protein has been linked to shortening of G1 (ohtsubo 1993), decreased requirement for growth factors (ohtsubo 1993), enhanced proliferation (bedrosian 2004), induction of chromosomal instability (Spruck, Akli 2004) and polyploidy (Lengauer lab FBW7/HCT ppr). These processes are felt to contribute towards the oncogenic potential pf cyclin E. The clinical relevance of cyclin E over expression is seen in multiple malignancies where cyclin E over expression is linked to poorer outcomes (Bani-Hani, Tissier, Ferreri, Rosen 2006, Farley, Keyomarsi NEJM, Porter 1997, Fukuse, Jang 1999, Mishina) , most notably in breast (Keyomarsi NEJM, Porter 1997) and ovarian cancer (Rosen 2006, Farley).

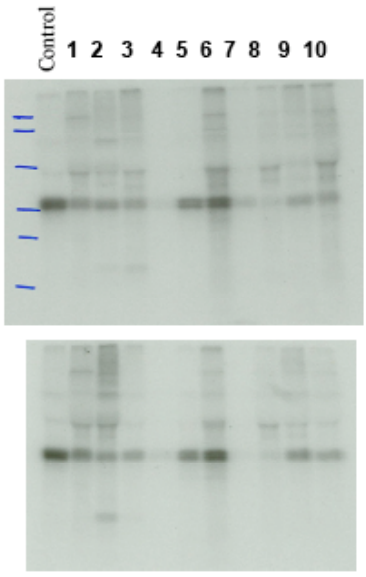
Although the prognostic role of cycin E is well established, and cyclin E has been shown to mediate resistance to anti-estrogens, there is limited information as to its role as a predictor of response to chemotherapy. We have previously reported that over expression of LMW cyclin E in an *in vitro* ovarian cancer model provides these cells with a proliferative advantage and resistance to inhibition by p21 and 27. LMW cyclin E overexpression in this model system also increased sensitivity to cisplatin treatment. We therefore hypothesized that cyclin E over expression, by abrogating the G1 checkpoint and increasing the proliferative fraction would make tumor cells more susceptible to S phase targeted therapies such as cisplatin. In addition, since the biologic activity of cyclin E is effected through is associated kinase activity, we further hypothesized that cyclin E over expression and thus, enhanced kinase activity, would also predict

for response to platinum based therapy. We tested this hypothesis in a cohort of 111 patients with advanced ovarian carcinoma who underwent platinum based chemotherapy at the University of Texas, M.D.Anderson Cancer Center.

Results

In the third year of the proposed grant we have completed the last aim/task of this grant application and will be submitting our work for publication.

Task 5: Collection and processing of ovarian tumor tissue



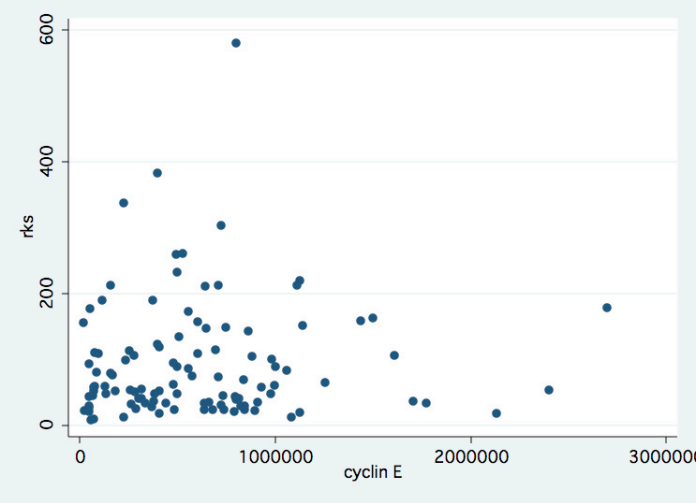
Samples	Week 1 (% of ctrl)	Week 2 (% of ctrl)	Mean	Standard deviation
Control	100	100	100	n/a
1	35	52	43.5	12
2	28	33	30.5	3.5
3	24	27	25.5	2.1
4	10	9	9.5	0.7
5	54	40	47	9.9
6	96	94	95	1.4
7	19	8	13.5	7.8
8	12	13	12.5	0.7
9	31	35	33	2.8
10	23	27	25	2.8

Determination of cyclin E associated kinase activity

Sufficient sample was available from 108 patients for cyclin E associated kinase evaluation. Cyclin E immmunoprecipitates from each tumor sample were assessed for cyclin E associated kinase activity using Histone H1 as substrate. Relative kinase activity for each sample was calculated as a percent of reference control sample. In order to ensure reproducibility of the assay, 11 samples (10%) were repeated at least twice. These samples were repeated on separate on separate days at least 1 week apart. The results of 10 of these samples are shown in Figure 1. Sample means and standard deviation were calculated

for each replicate sample and the co-efficient of variation was computed. The average co-efficient of variation was 9.9% indicating high reproducibility.

*Association between cyclin E expression and its associated kinase activity*We next examined the correlation between cyclin E expression level and its associated kinase activity in the 108 patients for whom both data points had been obtained. Cyclin E kinase assays were performed using Histone H1 as substrate. We found that cyclin E protein level in the tumor samples did not correlate with the level of cyclin E associated kinase activity (Figure 2) This result demonstrates the complexity of kinase activity which depends not only on the protein level of cyclin E, but also on the levels of its kinase binding partner, cdk2, and additionally on the expression of the natural inhibitors, p21 and p27. Since cyclin E activity did not appear to be linked to cyclin E protein levels, we next sought to assess the importance of each of these two parameters of cyclin E over expression on response to treatment to chemotherapy.



Response to chemotherapy

Clinical data on 110 patients was available to determine response to chemotherapy. Response to therapy was defined as per the GOG criteria. Patients who progressed on chemotherapy or who relapsed within 6 months of completing therapy were deemed to be non-responders. All other patients were categorized as responders to chemotherapy. Using this criteria, 48 patients were identified as non-responders and 63 were responders. In a univariate analysis, predictors of response to chemotherapy included optimal surgery, cyclin E and p27 expression levels (Table 1). All patients who responded had received optimal debulking. A significantly lower incidence of response was seen in patients who expressed high levels of cyclin E and among those who expressed high levels of p27 protein. Cyclin E associated kinase activity was not predictive of chemotherapy response.

We next attempted to perform multivariate logistic analysis of treatment response using the factors with univariate significance (optimal vs suboptimal surgery, cyclin E expression and p27 expression). Since all responding patients were limited to the optimally debulked group, we planned to perform the analysis in only the subset of patients with optimal surgery. However, only one factor was significant in this subgroup, cyclin E expression level, therefore no further analysis was possible.

Table 1

Variable	p-value
Age	0.058

Stage (III vs IV)	0.076
Histology (serous vs other)	0.763
Surgery (optimal vs suboptimal)	0.014
Cyclin E expression (high vs low)	0.017
Cyclin E associated kinase activity	0.288
p27 expression (high vs low)	0.046

DISCUSSION

Over expression of cyclin E has been reported in many human cancers (REF) and in many studies is linked to poor prognosis (REF). Less well known is the role of cyclin E as a marker of response to chemotherapy. Over expression of cyclin E is thought to result in enhanced activity of the cyclin E-cdk2 complex and a more efficient transition into S-phase. Therefore, cyclin E overexpressing cells would be expected to be more susceptible to agents that target S-phase such as cisplatin and carboplatin. In this study, we test this hypothesis and find that although cyclin E levels predict for response to chemotherapy, cyclin E associated kinase activity does not. Furthermore we find that it is low rather than high levels of cyclin E that predict for a population most likely to respond to platinum based therapy. These data therefore suggest that high levels

of cyclin E mediate alterations independent of its kinase regulatory function that leads to resistance.

Several lines of evidence suggest that cyclin E may have important biological roles that are independent of its ability to bind and activate cdk2. Whereas cdk2 has been shown to be dispensable for tumor formation in p27 null animals, cyclin E deficient cells are relatively resistant to oncogenic transformation (Geng, Cell 2003). More direct evidence comes from recent data that cyclin E has a centrosomal localization signal that is independent of its kinase function (Matsumoto, Science 2004). Results fromour study now provide translational support for the kinase dependent and kinase independent activities of cyclin E.

As agents targeting cyclin dependent kinases undergo clinical trials, the results from our study highlight that cyclin levels may not be an appropriate surrogate for cyclin associated kinase activity. For instance, cyclin E activity is dependent on multiple factors in addition to the level of cyclin E expression, including expression of cdk2, p21 and p27. It is the sum of these components that determines the ultimate activity of the cyclin E-cdk2 complex which mediates the transition across the restriction point into S-phase. Our data therefore suggests that therapies that target the cyclin dependent kinase activity may need to select patients on the basis of kinase function rather than expression of the target protein.

Conclusions

As presented in detail in this report-we have addressed task 5 of the grant application in the last 12 months.